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# International Journal of Environmental Health Research

Publication details, including instructions for authors and subscription information:

http://www.tandfonline.com/loi/cije20

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Version of record first published: 10 Jul 2012

To cite this article: Alexandre Naegele, Gabriel Reboux, Emeline Scherer, Sandrine Roussel & Laurence Millon (2012): Fungal food choices of Dermatophagoides farinae affect indoor fungi selection and dispersal, International Journal of Environmental Health Research, DOI:10.1080/09603123.2012.699029

To link to this article: <a href="http://dx.doi.org/10.1080/09603123.2012.699029">http://dx.doi.org/10.1080/09603123.2012.699029</a>



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## Fungal food choices of *Dermatophagoides farinae* affect indoor fungi selection and dispersal

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(Received 8 February 2012; final version received 30 April 2012)

House dust mite (HDM) feces and molds are the main allergens involved in allergic asthma. Differences exist between the housing fungal biome of allergic patients and standard or unhealthy housing. House dust mite (HDM) feed off spores and transport them on their bodies, but do they have fungal food preferences? We observed *Dermatophagoides farinae in vitro* with 16 mold menus and repeated the experiment 10 times. This observation led us to define *Alternaria alternata*, *Cladosporium sphaerospermum*, and *Wallemia sebi* as "tasty" molds and *Penicillium chrysogenum*, *Aspergillus versicolor*, and *Stachybotrys chartarum* as "repulsive" molds. The food preferences of *D. farinae* may play a role in the following two phenomena: a decrease in spore numbers due to HDM consumption and a scattering of spores that stick to the bodies of HDMs. The extent of these two phenomena should be estimated in future studies for other common domestic HDM species.

Keywords: Dermatophagoides farinae; fungi; spores; selection; dispersal

#### Introduction

House dust mite (HDM) feces and molds are the main allergens involved in IgE-mediated allergic asthma (Vandentorren et al. 2003; Fisk et al. 2007). *Dermatophagoides pteronyssinus* (Trouessart) and *Dermatophagoides farinae* (Hughes) are present in dwellings in variable proportions. *Dermatophagoides farinae* is found in mattresses, carpets, and soft furnishings, whereas *D. pteronyssinus* is found most frequently in mattresses. The concomitant presence of HDMs and molds increases the frequency and severity of asthma attacks (Prescott 2003). The level of total IgE is significantly higher in children exposed to both fungi and HDMs (Su et al. 2005). House dust mites (HDMs) contribute to mold transportation (McGinnis 2004). Differences exist between the housing fungal biome of allergic patients and unhealthy housing (Vesper et al. 2006; Bellanger et al. 2009; Reboux et al. 2009). House dust mites (HDMs) may modify patient exposure to allergens in dwellings by selecting and transporting some molds in the same way as astigmatic mites do in stored grain silo (Van Asselt 1999).

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This study aimed to determine the food preferences of *D. farinae* between six fungal species commonly found in housing and responsible for allergic diseases.

#### Materials and methods

#### **HDMs**

Dermatophagoides farinae were identified according to morphological and genotypical characteristics and by sequencing of internal transcribed spacer (ITS) regions. House dust mites (HDMs) were bred at 21°C and relative humidity at 45%. House dust mites (HDMs) were fed Saccharomyces boulardii, fish food, and beard hair (Unpublished data supplied by Christian Bories (Assistant Professor, University of Paris 11, Chatenay Malabry, France)).

#### Molds

Six common indoor molds were used as food for HDMs: *Alternaria alternata*, *Wallemia sebi*, *Cladosporium sphaerospermum*, *Stachybotrys chartarum*, *Penicillium chrysogenum*, and *Aspergillus versicolor*, respectively, registered in the BBCM-IHEM collection (22669, 16284, 18883, 22672, 22667, 22671).

#### Device and protocols

In a Petri dish, six "corridors" (35 mm  $\times$  10 mm) connected with a central sector (diameter: 15 mm), were cut from agar (agar 2%) (thickness: 2 mm) (Figure 1). At the corridor extremity, 500  $\mu$ l of agar was arranged to obtain six lenticular domes

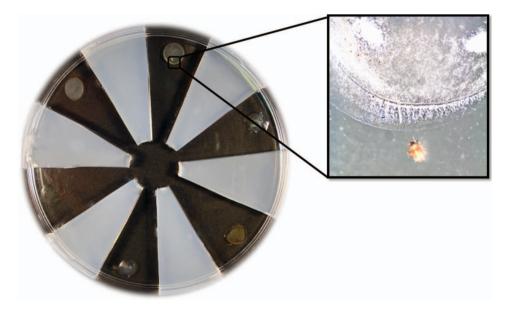


Figure 1. Six mold devices and HDMs eating *Wallemia sebi* spores. This device consists of six corridors cut from a gelose (Agar 2%) with six molds scattered on six lenticular domes and a central section for HDMs.

(diameter: 8 mm). Calibrated suspensions of  $10^7$  spores/mL were prepared for the six mold species. The domes were then seeded with  $100 \,\mu$ l of spore solution and incubated at  $30^{\circ}$ C for 7 days. Ten *D. farinae* individuals were then placed in the center of the device. House dust mites (HDMs) were observed in mold cultures through a binocular microscope with cold light (Leica S6D, Solms, Germany) after 180 min. This time span was determined by a preliminary test to establish HDM movement speed (7 mm/min.). Movement was preceded by an adaptation time due to the environmental change (breeding/Petri dish). Dust mites have to adapt to the space to turn to the tasty mold.

Each of the following tests was repeated 10 times (160 observations in all with 10 HDMs for each test). Only HDMs that were seen to consume mold were included in the analysis.

First, six molds were presented to HDMs in each device (Figure 1), in order to divide molds into two groups: "tasty fungi" and "repulsive fungi". Molds chosen at least once were considered tasty and those not chosen were considered repulsive.

Second, each tasty fungus (*Alternaria*|*Cladosporium*|*Wallemia*) was placed alongside each repulsive fungus (*Penicillium*|*Stachybotrys*|*Aspergillus*) in a different dish (nine dish combinations in total).

Third, the movement of *D. farinae* was studied with each of the six isolated molds. *Dermatophagoïdes farinae* chose whether or not to eat the mold.

#### Results

The results are summarized in Table 1.

The first experiment (six molds) (n = 10) enabled us to divide molds into two groups: tasty: 20% of deposited HDMs chose A. alternata, 13% C. sphaerospermum, 8% W. sebi – and repulsive: 0% for the other three species (P. chrysogenum, A. versicolor, and S. chartarum).

The second experiment (with two molds) (n = 30) confirmed this classification: 48% of HDMs chose A. alternata, 35% C. sphaerospermum, 33% W. sebi, 9.3% P. chrysogenum, 12% A. versicolor, and 13% S. chartarum.

The third experiment (n = 60) showed that this choice was "deliberate": 31% chose A. alternata, 36% C. sphaerospermum, 24% W. sebi, 7% P. chrysogenum, 3%

Table 1. Choice of molds consumed by D. farinae.

|   |           | Cladosporium<br>sphaerospermum | Wallemia<br>sebi | Penicillium<br>chrysogenum | 1 0       | Stachybotrys<br>chartarum |
|---|-----------|--------------------------------|------------------|----------------------------|-----------|---------------------------|
| 6  molds $(n = 10)$   | 2 (1.8)   | 1.3 (1.1)                      | 0.8 (0.7)        | 0 (0)                      | 0 (0)     | 0 (0)                     |
| ,   |           | Tasty fungi                    |                  | Repulsive fungi            |           |                           |
| 2 molds $(n = 90)$  | 4.8 (0.7) | 3.5 (0.7)                      | 3.3 (0.7)        | 0.9 (0.3)                  | 1.2 (0.2) | 1.3 (0.3)                 |
| $ \begin{array}{c} 1 \text{ mold} \\ (n = 60) \end{array} $ | 3.1 (0.7) | 3.6 (0.4)                      | 2.4 (0.8)        | 0.7 (0.4)                  | 0.3 (0.4) | 0.7 (0.4)                 |

Notes: n: test for each device. In bold: mean number of D. farinae choosing the fungi species of 10 individuals. In brackets: standard deviation.

A. versicolor, and 7% S. chartarum. When offered these latter three species, more than 90% of the HDMs preferred to go without food.

#### Discussion

Our observation of *D. farinae in vitro* together with 16 menus consisting of six molds, repeated 10 times, led us to define *A. alternata*, *C. sphaerospermum*, and *W. sebi* as "tasty" and *P. chrysogenum*, *A. versicolor*, and *S. chartarum* as "repulsive" molds. Only certain mold species were consumed by *D. farinae*.

House dust mites (HDMs) moved towards "tasty" mold species, ate them and disseminated the spores attached to their body during movements (Figure 2) as has been shown in astigmatid mites (*Acarus siro*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae*) (Hubert et al. 2004). In their study, the preferred mold species were *A. alternata*, *C. sphaerospermum*, and *W. sebi. Dermatophagoïdes farinae* were able to detect repulsive fungal species at a considerable distance: 45 mm (equivalent to 7 min of movement) and move further away. This suggests that *D. farinae* actively select molds.

A study on stored grain shows that tasty fungi as a food source for stored mites are more commonly dispersed than those not chosen. Fungal dispersions caused by mites depend on mite species (Hubert et al. 2003). This is also the case for plants (Gamliel-Atinsky et al. 2010), possibly through dispersal by an insect vector (Roets et al. 2009). However, the ability of mites to disperse specific fungal propagules remains questionable because it was not possible to prove selective eating and dissemination through the fecal pellets (Renker et al. 2005). The astigmatid mites degrade around 50% of spores. It is hard to determine if it is the eating or dispersal due to astigmatid mites which play the more important role (Nesvorna et al. 2012).

The large proportion of HDMs – more than 100 HDMs per grams of dust and more than 2 million per mattress (Korsgaard 1998) – suggests a potentially significant influence on fungal biome. The behavior of HDMs towards molds is modulated by the nature of fungi and the compounds they emit.

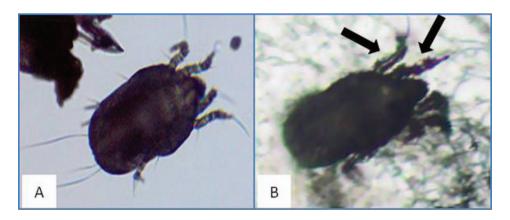


Figure 2. Observation of a *Dermatophagoides farinae* before (A) and after (B) consumption of *Wallemia sebi* spores and the girdle formed around the legs and body of the dust mites (Arrow) ( $\times$  40, binocular microscope with cold light, Leica S6D<sup>®</sup>, Solms, Germany).

An HDM prevention program (removing carpets, using anti-dust mite covers) modifies the indoor environment of allergic patients.

It is true that other factors are involved in the dispersal of fungi spores such as airflow, temperature, damp, and sometimes flooding. Research into mold exposure in asthma patients should examine the extent of the role of HDMs in destroying and dispersing mold colonies compared to the role of environmental factors.

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